3-1987

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Abstract

A new procedure for the quantification of soft lens deposits without causing any additional lens damage is investigated. The amount of a protein contaminant bound to a hydrogel lens is directly determined by subtracting the known mass before protein contamination, from its mass after protein contamination. 48 hydrophilic lenses (in varying conditions from unused to thoroughly spoiled, spherical and toric) were passively dehydrated and weighed with a mean standard error of 5.5 micrograms. Using this technique the weights of 8 unused 45% water Hydrocurve II and 8 unused 55% water lenses were determined. The lenses were contaminated by protein and their masses evaluated again. Both 45% and 55% groups showed significant increases in weight ($p>.01$) and the 55% group experienced a greater gain ($p>.01$).

Degree Type

Thesis

Degree Name

Master of Science in Vision Science

Committee Chair

James E. Peterson

Subject Categories

Optometry

WEIGHT QUANTIFICATION OF PROTEIN DEPOSITS ON HYDROGEL LENSES

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Submitted to the faculty of
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March 1987

ABSTRACT

A new procedure for the quantification of soft lens deposits without causing any additional lens damage is investigated. The amount of a protein contaminant bound to a hydrogel lens is directly determined by subtracting the known mass before protein contamination, from its mass after protein contamination. 48 hydrophilic lenses (in varying conditions from unused to thoroughly spoiled, spherical and toric) were passively dehydrated and weighed with a mean standard error of 5.5 micrograms. Using this technique the weights of 8 unused 45% water Hydrocurve II and 8 unused 55% water lenses were determined. The lenses were contaminated by protein and their masses evaluated again. Both 45% and 55% groups showed significant increases in weight ($p>.01$) and the 55% group experienced a greater gain ($p>.01$).

INTRODUCTION

Hydrophilic contact lenses, especially the extended wear variety, are becoming more and more popular, both as cosmetic alternatives to spectacle wear and tools used in the visual rehabilitation of post-surgical or otherwise compromised patients. A major obstacle, however, confronting both daily and extended wear varieties has been the affinity for deposits to develop on the lenses of many patients. McClure et. al. [1] showed measurable decreases in visual acuities of patients whose soft lenses were contaminated with protein deposits, while Giant Papillary Conjunctivitis (GPC) occuring among soft lens wearers has been associated with the presence of lens deposits[2,3]. In addition to various tear proteins[4,5], other tear constituents such as; calcium[6,7], lipids[8], sodium, phosphorous, potassium, and sulfur[9], have also been described as being responsible for lens spoilage. Additional sources of possible contamination are the environment (from airborne particulates ie. iron[10], nicotine, bacteria, molds, etc.), cosmetics[11] and chemical preservatives[12] (ie. chlorhexidine, benzalkonium chloride, thimersol, etc.) from various contact lens solutions.

Some of the many techniques used to identify the aforementioned agents included; thin paper chromatography, scanning and transmission electronmicroscopy, X-ray microanalysis, two-dimensional electrophoresis, mass and atomic absorption spectrometry, and interference microscopy. Each of these procedures required that lens materials be brought into contact with reagents and stains, or prepared in a manner which either destroyed the lenses outright or rendered them unfit for further use or testing.

Other studies, based more on clinical observations or less invasive types of microscopy failed to obtain quantifiable data. Levy[13] reported on the length of time (in weeks) required for calcium deposits to first appear on the lenses of 5 patients, and the frequency of lens replacement. Hathaway and Lowther[14] used a modified Rudko classification system to evaluate the heaviness of cultured deposits. Their system classified deposits as ranging from clean (under 7x magnification) to heavy (visible to the unaided eye), the extent of coverage (0% - 100% in 25% increments), and the overall physical appearance of the deposit.

The advent of newer, higher water content polymers and extended wear lens materials calls for the development of methods to quantify deposits, evaluate a material's affinity for deposits, and to test the effectiveness of cleaners. This experiment presents a new technique which objectively evaluates lens deposits on the basis of weight alone. By subtracting the known dry mass of a soft lens prior to contamination from its dry mass after contamination, it then becomes possible to quantify the amount of contaminant bound to a lens. Since every

addition in lens mass constitutes a source of possible spoilage or patient irritation, it is imperative (if we are to save the lens and reduce patient dissatisfication) that even small changes in mass be detected and removed as soon as possible.

METHODS

In order to reduce or remove loosely bound contaminants from the surface of the sample lenses, they were cleaned using a Barnes Hind Hydra-mat and Softmate ps* weekly cleaner (according to package directions). To insure the relative absence of cleaner or saline from the lens matrix each lens was allowed to equilibrate in distilled water for 24 hours at a constant temperature of 35 degrees C.

Snyder and Koers[15] describe a dehydration process in which a lens is placed in an open top dessicating chamber, inside a vacuum oven at 100 degrees C. and 40mmHg partial pressure for 24 hours. The vacuum was released by drawing air through a dessicant-filled cylinder and the lenses were weighed within 20 seconds of removal from the oven. This procedure, however effective, relies on temperatures which exceed the manufacturer's maximum recommendations for many (if not most) of the hydrogel materials .

For this experiment, dessicating chambers (fig.1) were prepared by filling 16 one pint, airtight, resealable containers with one inch of Drierite (indicating blue, anhydrous $CaSO_4$ from Hammond Laboratories). Using lens tweezers, each lens was set into an open lens case suspended by a mesh shelf two inches above the dessicant. The chamber was sealed and stored for 24 hours at 35 degrees C. to allow the lenses to dehydrate. (From comparison with measurements taken at 6,12,24,48,and 72 hours, it was determined that no significant drying occurred after 24 hours.) The lenses were removed from their chambers and immediately weighed on a Sartorius A - 200S microbalance. To obtain baseline measurements, 48 contact lenses (16 unused, 16 used but with no visible deposits, and 16 used and heavily contaminated lenses) were dehydrated, weighed, and rehydrated in distilled water for 24 hours at 35 degrees C. ten times each (table 1).

After recording baseline measurements, the 16 unused lenses (8 hydrocurve II 55% and 8 Hydrocurve II 45% water lenses) were immersed for six hours at 35 degrees C. in a solution containing commercially available albumin* (at twice the normal ocular concentration, 788mg/100ml), lysozyme** (at twice normal ocular concentration, 430mg/100 ml) and normal

*albumin, fraction V, 96 - 99%, Sigma No. A - 4503

**chicken egg white, grade I, 3x crystallized, Sigma L-6876

saline, buffered to pH of 7.2 . Each lens was removed from solution, placed in a Hydra-mat cleaning unit filled with unpreserved saline, and spun for thirty seconds in clockwise - counterclockwise rotations. Next they were dipped in distilled water to remove the saline rinse, then placed in the dessicating chambers to dehydrate. The lenses were dehydrated and weighed one time as previously described.

RESULTS

The results of the ten baseline measurements from all 48 lenses (table 1) show both repeatability and a distribution which approximates the normal. The values for standard error of the estimate range from a low of 2.7 x10-5g. to a high of 9.0x10-5g with a mean standard error of 0.55 x 10-5g., of which is less than the sensitivity of the balance.

The average variance for each group of lenses, unused, used with both light and heavy degrees of contaminations, was calculated and compared through F-ratios (15 degrees of freedom in both numerator and denominator). The lack of significant difference between the groups at the 0.01 level indicates homogeneity of variance through the entire sample.

F = mean variance of group 1/mean variance of group 2 = 3.8709/3.5522 = 1.0897*
F = mean variance of group 1/mean variance of group 3 = 3.8709/2.0913 = 1.8509*
F = mean variance of group 2/mean variance of group 3 = 3.5522/2.0913 = 1.6985*
Fcv(15,15) = 3.41
*no significance at .01, .05, or .1 levels

The pre- and post- contamination data (table 2) was compared by a two factor repeated measures ANOVA*(table 3) and the following was found to be significant; 1) the increase in weight of all lenses after protein contamination ($p>.001$), 2) a difference in the amount of weight increase between the 45% water and 55% water lenses ($p>.001$), and 3) a definite interaction effect between water content and amount of weight increase ($p>.001$). One way ANOVA's (table 4) performed on the 45% and 55% lens groups showed both groups to increase in weight (using the conservative Scheffe post hoc criteria, at $p>.01$), and a paired t - test (dependent samples) showed the 55% water lenses to form heavier deposits than the 45% lenses ($p>.001$).

DISCUSSION

Since no prior history of work in this area was found, lenses with different parameters

*statistics by Statview 512+, software for Apple Macintosh, Abacus Concepts Inc. 1986.

(water content, OAD, thickness, polymer type, and varying degrees of contamination) were repeatedly tested in order to show both reliability and precision in the dehydration and weighing processes.

Although the customary method used for putting deposits on hydrogel lenses calls for the cycling of lenses through as many as 60 heat ascepticization and inoculation procedures, an alternative method, lens immersion in a protein solution for six hours at 35 degrees, still allowed deposit formation without undo exposure at high temperatures.

From the data on the 45% lens group it is clear that even small increases (on the order of tenths of a milligram) in mass are detectable. The accuracy of this test procedure was further demonstrated by the fact that none of the post- measurements showed a decrease in mass (even though one lens had no measurable change and another a much smaller than expected increase.)

CONCLUSION

The importance of being able to conduct testing such as this is obvious when considering that the lenses suffer no lasting effects from the procedure. In addition to testing different materials, this method could probably be applied to evaluate the effectiveness of different cleaners and cleaning regimens (since even very small mass changes are detectable). This method of analysis also has the distinct clinical advantage over others by making it possible to follow changes in lens mass over time, without destroying a patient's lenses.

DESSICATING CHAMBER APPARATUS
figure 1

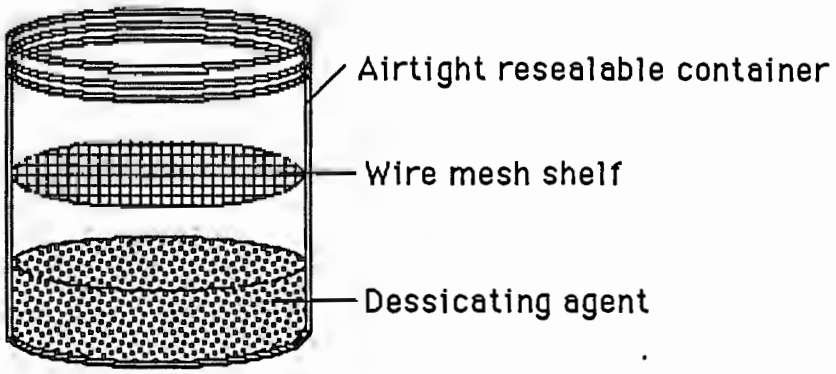

Table 1
Initial weights of all lenses

All measurements in ten thousandths of one gram (0.0001 g.)

lens type	lens #											Avg.	St. dev.	St. error
heavy dep.	1	153	152	151	148	149	150	151	153	150	149	150.60	1.71	0.54
heavy dep.	2	180	186	185	181	183	184	182	179	181	182	182.30	2.21	0.70
heavy dep.	3	189	193	188	194	194	191	191	194	192	190	191.60	2.17	0.69
heavy dep.	4	185	180	180	178	176	182	183	179	180	181	180.40	2.55	0.81
heavy dep.	5	200	196	198	201	197	200	202	199	200	201	199.40	1.90	0.60
heavy dep.	6	206	200	204	204	200	202	205	203	200	201	202.50	2.22	0.70
heavy dep.	7	121	120	121	119	118	119	120	120	117	119	119.40	1.26	0.40
heavy dep.	8	400	397	395	395	396	398	396	395	398	394	396.40	1.84	0.58
heavy dep.	9	118	119	120	118	116	120	115	118	119	118	118.10	1.60	0.50
heavy dep.	10	137	138	137	141	136	140	136	137	140	139	138.10	1.79	0.57
heavy dep.	11	212	209	212	209	209	210	208	213	213	210	210.50	1.84	0.58
heavy dep.	12	294	287	290	292	290	291	294	289	291	292	291.00	2.16	0.68
heavy dep.	13	261	269	264	264	263	264	266	265	268	266	265.00	2.36	0.75
heavy dep.	14	347	350	350	345	345	347	347	349	349	348	347.70	1.83	0.58
heavy dep.	15	229	229	229	227	224	226	229	230	228	226	227.70	1.89	0.60
heavy dep.	16	270	265	270	268	269	267	266	267	268	270	268.00	1.76	0.56
light dep.	17	259	256	260	260	259	260	261	259	261	260	259.50	1.43	0.45
light dep.	18	275	272	276	271	272	274	275	276	272	273	273.60	1.84	0.58
light dep.	19	318	313	321	322	323	318	318	320	317	319	318.90	2.85	0.90
light dep.	20	301	297	301	298	300	301	299	300	302	301	300.00	1.56	0.49
light dep.	21	240	240	241	240	237	242	239	241	240	240	240.00	1.33	0.42
light dep.	22	332	328	330	330	327	329	329	328	332	330	329.50	1.65	0.52
light dep.	23	261	263	261	261	257	258	262	263	261	260	260.70	1.95	0.62
light dep.	24	229	234	230	236	235	230	234	233	234	232	232.70	2.36	0.75
light dep.	25	271	269	270	270	271	272	271	271	270	273	270.80	1.14	0.36
light dep.	26	337	336	340	339	338	341	339	336	338	340	338.40	1.71	0.54
light dep.	27	190	189	190	193	189	190	192	189	188	190	190.00	1.49	0.47
light dep.	28	228	221	226	229	225	224	226	225	228	224	225.60	2.37	0.75
light dep.	29	337	338	331	335	334	336	338	335	336	336	335.60	2.07	0.65
light dep.	30	237	237	238	238	233	236	238	239	238	235	236.90	1.79	0.57
light dep.	31	358	353	352	356	351	353	356	354	354	355	354.20	2.10	0.66
light dep.	32	317	322	318	320	322	322	321	320	321	319	320.20	1.75	0.55
Unused 55%	33	182	184	183	184	179	181	182	184	182	181	182.20	1.62	0.51
Unused 55%	34	164	165	166	164	162	164	165	167	166	164	164.70	1.42	0.45
Unused 55%	35	180	180	183	180	180	181	180	182	183	181	181.00	1.25	0.39
Unused 55%	36	196	196	198	200	197	197	199	198	199	201	198.10	1.66	0.53
Unused 55%	37	184	184	186	184	183	184	185	184	183	186	184.30	1.06	0.33
Unused 55%	38	199	200	198	200	200	199	199	200	200	201	199.60	0.84	0.27
Unused 55%	39	173	172	171	174	171	170	172	172	170	169	171.40	1.51	0.48
Unused 55%	40	231	235	233	234	232	232	231	236	233	235	233.20	1.75	0.55
Unused 45%	41	405	408	407	404	406	409	406	403	407	405	406.00	1.83	0.58
Unused 45%	42	384	382	384	383	384	386	385	385	384	383	384.00	1.15	0.37
Unused 45%	43	310	312	310	309	310	309	310	312	310	311	310.30	1.06	0.33
Unused 45%	44	406	404	408	402	405	403	405	407	404	405	404.90	1.79	0.57
Unused 45%	45	381	379	382	378	381	381	379	380	380	381	380.20	1.23	0.39
Unused 45%	46	360	359	362	358	361	360	359	359	360	362	360.00	1.33	0.42
Unused 45%	47	255	249	252	251	253	255	251	252	254	252	252.40	1.90	0.60
Unused 45%	48	348	345	346	346	347	345	346	347	344	347	346.10	1.20	0.38

Table 2

Lens type	Lens#	Avg. Wt. before	Wt. After	Difference
Unused 55%	33	182.20	201.00	18.80
Unused 55%	34	164.70	190.00	25.30
Unused 55%	35	181.00	182.00	1.00
Unused 55%	36	198.10	214.00	15.90
Unused 55%	37	184.30	204.00	19.70
Unused 55%	38	199.60	220.00	20.40
Unused 55%	39	171.40	194.00	22.60
Unused 55%	40	233.20	260.00	26.80
Unused 45%	41	406.00	410.00	4.00
Unused 45%	42	384.00	387.00	3.00
Unused 45%	43	310.30	313.00	2.70
Unused 45%	44	404.90	410.00	5.10
Unused 45%	45	380.20	383.00	2.80
Unused 45%	46	360.00	360.00	0.00
Unused 45%	47	252.40	254.00	1.60
Unused 45%	48	346.10	349.00	2.90

Table 3

For a 2-Factor repeted measures ANOVA

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Lens (A)	1	200091.38	200091.38	60.783	0.0001
Subjects w/ groups	14	46086.509	3291.893		
Repeted Measure (B)	1	930.961	930.961	56.014	0.0001
AB	1	515.205	515.205	30.999	0.0001
B x subjects w/ groups	14	232.684	16.62		

Table 4

ONE FACTOR ANOVA-REPEATED MEASURES FOR 55% WATER LENSES

SOURCE:	df:	Sum of Squares	Mean Square:	F-test:	P value:
Between subjects	7	7100.829	1014.404	4.947	0.0194
within subjects	8	1640.295	205.037		
treatments	1	1415.641	1415.641	***44.11***	***0.0003***
residual	7	224.654	32.093		
Total	15	8741.124			

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:
AFTER vs. BEFORE	18.812	9.913*	44.11*

* Significant at 99%

ONE FACTOR ANOVA-REPEATED MEASURES FOR 45% WATER LENSES

SOURCE:	df:	Sum of Squares	Mean Square:	F-test:	P value:
Between subjects	7	38985.679	5569.383	1155.623	0.0001
within subjects	8	38.555	4.819		
treatments	1	30.526	30.526	***26.612***	***0.0013***
residual	7	8.029	1.147		
Total	15	39024.234			

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:
AFTER vs. BEFORE	2.763	1.874*	26.612*

* significant at 99%

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We would like take this opportunity to acknowledge the following people whose contributions and assistance made this research possible:

Dr. James Currie and Dr. William Jordan of the Pacific University Chemistry Deptartment for their time and technical assistance.

Mr. Ron O'Hara, sales representitive for Barnes Hind / Hydrocurve Inc. for supplying the lens materials used in this research.

A. Richard Reinke, O.D. Research Coordinator for Pacific University College of Optometry.

James E. Peterson, O.D. for his time and technical guidance from inception to completion.